Developments and Microbiological Applications in African Foods: Emphasis on Nigerian Wara Cheese

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I Raheem, D., 1997.
Transfer of Technology from North to South – Meeting the needs of a rural development: A review of the Mtwara-Lindi water project. Environmental Management and Health Journal, 8 (4): 124 – 127.

II Raheem, D., 1995.
Effect of amylase treatment on the consistency of oat bran porridge. International Journal of Food Science and Nutrition, 46 (3): 189-193.

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Foods from cassava and their relevance to Nigeria and other African countries. Journal of Agriculture, Food and Human Values, 18 (4): 383-390.

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ABSTRACT

African indigenous foods have received limited research. Most of these indigenous foods are fermented and they form part of the rich nutritional culture of many groups in African countries. The industrialization and commercialisation of these indigenous African fermented foods should be preceded by a thorough scientific knowledge of their processing which can be vital in the elimination of hunger and poverty. This study highlighted emerging developments and the microbiology of cereal-based and cassava-based food products that constitute a major part of the human diet in most African countries. In addition, investigations were also carried out on the coagulant of the *Calotropis procera* plant used in traditional production of Nigerian Wara cheese and on the effects of adding a nisin producing *Lactococcus lactis* strain originating from human milk to Nigerian Wara cheese.

Fermented cereal-based food such as ogi utilize popular African and readily available grains – maize, millet or sorghum as substrates and is popular as a weaning diet in infants. In this study, the bulkiness caused by starch gelatinization was solved by amylase treatments in the investigation on cooked and fermented oat bran porridge. A similar treatment could reduce the viscosity of any cereal porridge.

The properties of the Sodom apple leaves (*Calotropis procera*) extract in cheesemaking were studied. *C. procera* was affected by monovalent (K^+ and Na^+) and divalent (Mg^{2+} and Ca^{2+}) cations during coagulation. The rennet strength of this coagulant was found to be 7 % compared to animal rennet at 35 °C. Increasing the incubation temperature to 70 °C increased the rennet strength 28-fold. The molecular weight of the partially purified protease was determined by SDS-PAGE and was confirmed by Zymography to be approximately 60 kilodaltons. The high proteolytic activity at 70 °C supported the suitability of the protease enzyme as a coagulant in future commercial production of Nigerian Wara cheese. It was also possible to extend the shelf life of Wara cheese by a nisin producing lactic acid bacteria *Lactococcus lactis* LAC309. The levels of nisin in both whey and curd fractions of Wara were investigated, results showed a 3 log reduction of toxicogenic *Bacillus licheniformis* spiked on Wara after 3 days.

These studies are the first in Finland to promote the advancement of scientific knowledge in African foods. Recognizing these indigenous food products and an efficient transfer of technology from the developed countries to industrialize them are necessary towards a successful realization of the United Nations Millenium Development Program.

ABBREVIATIONS

APCTT	Asian and Pacific Centre for Transfer of Technology
AOAC	Association of Official Analytical Chemists
BSA	bovine serum albumin
BGG	bovine gamma globulin
κ-casein	a gene product of bovine casein (calcium insensitive)
CCP	colloidal calcium phosphate
CIAT	Centro Internacional de Agricultura Tropical
CFU	colony forming units
DNA	deoxyribonucleic acid
DTT	dithiothreitol
ECOSOC	Economic and Social Council of the United Nations
EDTA	ethylenediaminetetracetic acid
e.g	*exempli gratia*, for example
FAO	Food and Agriculture Organization
FPLC	Fast Protein Liquid Chromatography
IDF	International Dairy Foundation
i.e *id est*,	that is
Ig	immunoglobulins in milk
ILCA	International Livestock Centre for Africa
IITA	International Institute of Tropical Agriculture
kDA	kilodaltons
LAB	Lactic acid bacteria
LB	Luria Bertani medium
NPN	Non Protein Nitrogen
PCA	Plate Count Agar
PMSF	Phenylmethylsulfonyl fluoride
RFU	Relative fluorescence unit
RNA	riboxynucleic acid
SMS	Stable Micro Systems
TEMED	tetramethylene diamine
TSA	Tryptic Soy agar
UNIDO	United Nations Industrial Development Organization
UNCTAD	United Nations Conference on Trade and Development
VRBA	Violet Red Bile agar
WHO	World Health Organisation
YGC	Yeast Glucose Chloramphenicol agar

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1. INTRODUCTION

Indigenous foods are produced in homes, villages, and small cottage industries at prices within the means of a majority of consumers in the developing world. Hunger and poverty are common in this world where vast millions must support their families on less than US$1 per day (Steinkraus, 1983). An examination of these foods will provide clues as to how food production and preservation can be expanded thereby contributing to improved nutrition and reduction of poverty in African countries.

The past few decades have witnessed an interest in the indigenous foods of Africa. This interest has been triggered by the recurrency of famines that plagued the continent during that period. Indigenous knowledge in African agriculture, famine foods as well as fermented and non-fermented foods and the possibilities of modernizing their production are today among the important topics discussed concerned circles. But how can one improve these foods if one does not know what the actual foods are? The starting point of improvement is the acquisition of the complete indigenous knowledge concerning these foods. It remains a fact that to date, in spite of all the talk and concern we still know very little about what the very poor among the Africans – the sector most dramatically affected by famines – eat (Dirar, 1993).

In order to improve and increase the availability of food in sub-Saharan African countries knowledge of foods indigenous to this region is important. The application of science and technology to food processing in this region is hampered by limited research and misplaced priorities. Microbiological processing is an age long tradition in food production but there are few documented studies on African foods. This dissertation reviews foods native to sub-Saharan Africa such as cereal porridge, cassava root crop and cheese. In addition, the utilization of *Calotropis procera* (sodom apple extract) as a coagulant in Nigerian Wara soft cheese was investigated as well as the effects of including a nisin producing strain in the Wara soft cheese.

1.1 Cereal porridge in African diet

Cereal grains (sorghum or guinea corn, maize, rice and millets) are the most important substrates for fermented foods in sub-Saharan Africa. They are fermented to produce a variety of foods ranging from alcoholic and non-alcoholic beverages, porridges, dumplings and baked products (Muller, 1980).

Cereal porridge has an international acceptance in many regions of the world. In tropical African countries several fermented and unfermented cereal foods are consumed daily and they form an essential part of the diet (Hesseltine, 1979). Despite the dawn of science and technology in Africa, the production of fermented cereal foods is still largely a traditional family art done in crude manner. The production has not increased substantially and shelf lives are often short (Odunfa, 1985).

Traditional cereal foods can either be liquid or solid type, the liquid type is free flowing thin porridges or beverages while the solid types are very stiff dumplings used as a heavy meal. Some traditional fermented cereal foods and their regions of origin are shown in Table 1.

Table 1. Classification of some traditionally fermented cereal foods

Product	Substrate	Region
Liquid type		
Ogi	maize, sorghum, millet	Nigeria
Koko	maize, sorghum, millet	Ghana
Uji	maize, sorghum, millet	East Africa
Mahewu	maize meal, wheat flour	South Africa
Atole	maize, sorghum	Central America
Sowen	oatmeal, buttermilk	Scotland
Kiesa	oat	Karelia, Finland
Solid type		
Agidi	maize ogi slurry	Nigeria
Kenkey	maize	Ghana
Mawe	maize	Benin
Ugali	maize	East Africa
Idli	dehulled rice	India

(Hesseltine 1979)

Liquid type porridges contain more than 90 % water and are usually eaten with a spoon, many are sour porridges. The cereal grains are fermented and milled to produce a gruel which is known by various names in different parts of Africa. Ogi is a major staple food widely taken in Nigeria, and a traditional infant weaning food.

Lactic acid bacteria play a major role in fermentation of grains. A summary of predominant lactic acid bacteria present in different African fermented cereal porridge are shown in Table 2.

During the preparation of ogi, predominant fungal flora of *Aspergillus, Penicillum*, and *Fusarium* species are eliminated in the early steeping period. After 24 h of steeping, the predominant microbes include *Lactobacillus plantarum, Aerobacter cloacae* and *Corynebacterium.* The pH of the steeping liquid decreases to about 4 which give rise to conditions favourable for subsequent souring of the maize mash by *L. plantarum* and yeasts *Saccharomyces* spp., *Candida mycoderma* and *Rhodotorula* (Odunfa, 1985; Olasupo et al. 1996).

The dough from koko has been shown to contain mainly yeasts and lactic acid bacteria. They are homofermentative *Pediococcus cerevisiae* and heterofermentative *Leuconostoc mesenteroides* and *Leuc. fermenti,* while the sour water obtained from koko is dominated by *Weisella confusa* and *L. fermentum* (Lei and Jakobsen 2004). The predominant micro-organisms such as *L. fermentum*, *Saccharomyces cerevisiae* and *Candida krusei* in fermented maize dough can be used as starter cultures to modify the aroma of fermented maize dough (Annan et al. 2003).

Uji fermentation is characterised by the sequential growth of the dominant microorganisms where initially coliforms grow rapidly and are later succeeded by the lactic acid bacteria. Early acid production at high rates by the lactic acid bacteria rapidly restricts

Table 2.

Product	Lactic acid bacteria	Reference
Nigerian Ogi	*Lactobacillus plantarum*, *L. brevis, L. renteri, L. delbrueckii, L. casei*, *L. acidophilus*	Banigo et al. 1977; Odunfa,1985; Sanni et al. 1999.
Beninese Mawe	*Lactobacillus, brevis, L. fermentum, L. reuteri, L. curvatus, L. confuses, L. buchneri, L. lactis, L. salivarius, L. celloboisus, Pediococcus acidilactici, P. pentosaceus, Leuconostoc mesenteroides*	Hounhouigan, 1994; Louembe et al. 2003.
Kenyan Uji	*Leuc. mesenteroides, L. plantarum, L. fermentum, L. cellobiosus, P. acidilactici, P. pentosaceus, Lactococcus spp.*	Mbugua, 1981; Gatumbi and Muriru, 1983;
South African Mahewu	*L. brevis, L. bulgaricus, L. acidophilus, Lact. lactis*	Schweigart & de Wit, 1960; Schweigart & Fellinghan, 1963; Hesseltine, 1979
Ghanaian Kenkey	*L. brevis, Lactobacillus sp.*	Nyako, 1977; Halm et al. 1993; Haard et al. 1999.

coliform activity thereby eliminating the problems of off-flavours and flavour instability. *L. plantarum* is mainly responsible for souring of uji although early activity of heterofermentative strains of *L. fermentum*, *L. cellobiosus* and *L. buchneri* during the fermentation is evident (Mbugua, 1981).

The main microorganism in native mahewu is *Lactococcus lactis.* Mahewu is now manufactured on a modern industrial scale as a dry product by Jabula Foods Limited in South Africa. This has led to the development of starter cultures. *L. delbrueckii* and *L. bulgaris* are used at 50 ºC to produce an acceptable mahewu. The elevated temperature inhibits undesirable bacteria (Odunfa, 1985).

One of the major challenges highlighted in the formulation of a weaning cereal food porridge is its bulkiness caused by starch gelatinization when cooked. Such a food should have a lower viscosity i.e. easy to swallow especially for infants (Nout, 1993).

This problem can be solved by treatments with amylase, which would solubilize starch and reduce gel viscosity (Desikachar, 1980). Apart from low viscosity the malted weaning food can be expected to permit better digestibility of the starch, as partial starch breakdown to dextrins occur during malting. Lowered starch complexity and partial predigestion by enzymes should help in its utilization by a child being weaned from a lactose based milk diet to a starch based cereal diet (Malleshi and Desikachar, 1980).

A product rich in soluble fiber with higher energy values and a sufficiently lower consistency that is spoonable was possible with cooked, fermented oat bran porridge (Salovaara et al. 1990). A similar concept can be employed in the formulation of new products from cooked and fermented porridge in the African cereal diet on a large scale production. Desirable technological, organoleptic and nutritional properties in any cereal based end product are highly dependent on proper gelatinisation of its starch. Above a certain temperature the starch granules swell and their structure is altered (Greenwood, 1976; Lund, 1981; Marconi et al. 2004).

Many foods are processed exploiting hydrolytic enzymes such as proteases, amylases and cellulases leading to partial breakdown of proteins, starch and cellulose (Cheetham, 1993; Wiseman et al. 1998). Modifications of starch paste viscosity can be accomplished by suitable adjustment of the processing conditions such as the enzymatic modification of the starch by amylase (Lineback and Wongsrikesern, 1980). Investigations on amylolytic activity confirmed *L. fermentum* OgiE1 as having a high yield of amylase production and being tolerant of a low pH of 2 thus having a potential benefit in lowering consistency in weaning foods (Sanni et al. 2002).

The soluble fiber and beta glucan contents of oats have been shown to have a significant effect on the reduction of serum levels of cholesterol, triglycerides and blood glucose. Therefore, there has been emphasis on research on the utilisation of oats (Behall et al. 1997; Bell et al. 1997). The beneficial effects of oat to cardiovascular health has dramatically improved its end-use value into a limitless range of products.

Generally, cereals can be used as fermentable substrates for the growth of probiotic microorganisms. The main parameters that have to be considered are the composition and processing of the cereal grains, the substrate formulation, the growth capability and productivity of the starter culture, the stability of the probiotic strain during storage, the organoleptic properties and the nutritional value of the final product (Charalampopoulos, 2002; Angelov et al. 2005). It has been proposed that an aggressive approach is needed to open new opportunities for enzyme and microbial applications that can benefit the food industry (James and Simpson, 1996). Additionally, cereals can be used as sources of nondigestible carbohydrates that besides promoting several beneficial physiological effects can also selectively stimulate the growth of lactobacilli and bifidobacteria present in the colon and act as prebiotics (Charalampopoulos et al. 2002).

1.2 Cassava root crop in African diet

Cassava (*Manihot esculenta*, Crantz) also called manioc, tapioca or yuca is a staple food for as many as 300 million Africans. It is the main famine food reserve grown in a broad belt across the continent (Burrell, 2003). Cassava ranks very high among crops that convert the greatest amount of solar energy into soluble carbohydrates per unit of area. The

carbohydrate production of cassava is about 40% higher than rice and 25% more than maize (Tonukari, 2004).

It is expected that sub-Saharan Africa will experience the most rapid growth in food demand with root and tubers averaging 2.6% per year through the year 2020 (Scott et al., 2000). This growth will account for nearly 122 million metric tonnes with most of the increase coming largely from cassava at 80 million metric tonnes (66% of the total). The total production of cassava would reach 168 million metric tonnes by 2020 based on the current production rate. It is recognised that the production of cassava has a great potential for providing raw materials that can meet the food needs of an increasing urban population if adequate support is given. The major beneficiaries of a more diversified world market for cassava will be for sub-Saharan African countries (Adeniji et al. 1997).

The nutrient composition of fresh cassava roots is compared to some other staple foods common in West Africa (Table 3). Cassava has a high energy value and high vitamin C content. These values will vary upon processing when the water content is low after heat treatment. Cassava is low in niacin, but the leaves from cassava have higher nutrient values.

There are several food products which are processed traditionally from cassava which can be further developed by microbiological application (Balagopalan et al. 1988). One of the most popular fermented cassava root product is gari, which is eaten in a similar way to couscous or boiled rice. It requires no boiling but is moistened with hot water to the required consistency. The fermentation of cassava during gari production is anaerobic and exothermic. It is a two stage process (Collard and Levi, 1959; Kobawila et al., 2005). In the first phase, *Corynebacterium manihot* attacks the starch of the root leading to the production of various organic acids such as lactic and formic. In the second phase, the acidic condition stimulates the growth of a mould, *Geotrichum candida* which begins to proliferate rapidly bringing about further acidification and the production of a variety of aldehydes and esters that are responsible for the taste and aroma of gari.

The bacterial population in ntoba mbodi, a Congolese alkaline fermented cassava leaf product, is essentially lactic acid bacteria notably *L. plantarum*, *L. fermentum*, *Lactococcus lactis* and *Pediococccus cerevisiae* (Kobawila et al., 2005).

Cassava leaves are rich in proteins (26 - 42% of the dry weight) as determined in 181 varieties (IITA, 1974; West, 1988), and rich in minerals and vitamins. Much of cassava´s protein is made up of the enzyme linamarase and its activity has been found to be about 200 times greater in the leaves than in the roots (Bokanga, 1995). The leaves contain most essential amino acids but are limited in phenylalanine and sulphur containing amino acids such as methionine (Ravindran et al. 1988; Diasolua et al. 2003). These amino acids are essential for the detoxification of the residual cyanogens present in insufficienly processed cassava roots (Gomez et al., 1985). It has been shown that cyanide is detoxified in the body by conversion to thiocyanate which is a sulphur containing compound. In normal health, a dynamic equilibrium between cyanide and thiocyanate is maintained, but in a low protein diet particularly one deficient in sulphur containing amino acids will decrease the detoxification capacity making a person more vulnerable to the toxic effect of cyanide (Oke, 1973; Cliff et al. 1985). The essential substrates for the conversion of cyanide to thiocyanate are thiosulphate and β-mercaptopyruvate which

Table 3. Average nutrient composition (per 100 g edible portion) of fresh cassava compared to that of some staple food crops in West Africa

	Food energy (calories)	Water (g)	Carbo-hydrate (g)	Protein (g)	Fat (g)	Calcium (mg)	Iron (mg)	Vit. A (IU)	Thiamine B1(mg)	Riboflavin B2 (mg)	Niacin (mg)	Vitamin C (mg)
Potatoes	82	78	18.9	2.0	0.1	8	0.7	tr	0.10	0.03	1.4	10
Sweet potatoes	117	70	27.3	1.3	0.4	34	1.0	500	0.10	0.05	0.6	23
Fresh cassava	146	62.5	34.7	1.2	0.3	33	0.7	tr	0.06	0.03	0.06	36
Yams	105	72.4	24.1	2.4	0.2	22	0.8	tr	0.09	0.03	0.5	10
Taro	104	72.5	24.2	1.9	0.2	23	1.1	tr	0.15	0.03	0.9	5
Maize	363	12	71	10	4.5	12	2.5	tr	0.35	0.13	2.0	0
Sorghum	335	12	71	10.4	3.4	32	4.5	tr	0.5	0.12	3.5	0
Cowpea	340	10	60	22	1.5	90	5.0	20	0.9	0.15	17.0	tr

tr, trace amount (Latham, 1969; Okigbo, 1980)

are derived mainly from sulphur containing amino acids, cysteine and methionine (Fiedler and Wood, 1956; FAO, 1990).Therefore, it is important to supplement cassava rich diet with cereals and legumes which will compensate for these limited amino acids.

The aqueous and ethanolic extracts of raw cassava tuber contain alakaloids, flavonoids, tannins, reducing sugars and anthocyanosides but do not contain cardiac glycosides, anthraquinone, phlobatinnins and saponins, while raw cassava leaves are characterised by the presence of alkaloids, flavonoids, tannins, anthraquinone, phlobatinnins, saponins, reducing sugars and anthrocyanosides but do not contain cardiac glycosides (Obuehi et al. 2005).

Cassava roots and leaves contain cyanogenic glucosides: linamarin and lotaustralin, which upon hydrolysis produce free hydrocyanic acid (HCN) which is poisonous and is a cause for concern (Mlingi et al. 1991). When cyanide enters the bloodstream it is converted by the enzyme rhodanase to thiocyanate, a sulphur containing compound. This compound can be deleterious by using up body sulphur in detoxification, thereby increasing the body's demand for sulphur containing amino acids or by interfering with the iodine uptake of the thyroid resulting in goiter. The evidence for an etiological role of high cyanide and low sulphur intake in Konzo (a paralytic disease) is now strong enough to urge prevention by promotion of efficient processing of cassava roots (Tylleskär, 1994). However, it should be emphasized that for millions of consumers, well processed cassava as a staple food has no negative effects (Bokanga, 1995).

Linamarin constitutes 90 % of the cyanogenic glucosides in cassava and the remainder is lotaustarin which has an additional methyl group on the carbon atom (Dunstan et al. 1996; Montgomerry, 1980). Cassava can be classified according to the level of hydrocyanic acid: very toxic variety with more than 100 mg HCN/kg of pulp, moderately toxic variety with 50 - 100 mg HCN/kg of pulp and not toxic variety with less than 50 mg/kg of pulp (Kobawila et al., 2005).

Hydrolysis occurs when the glucosides come into contact with the endogenous enzyme linnamarase and HCN is released as a result of cellular structure damage or when the root is crushed (Agbor-Egbe et al. 1995). The grating of cassava during which the cells are ruptured and subsequent processing such as fermentation and frying reduce the cyanogenic glycosides to safe levels (Dunican, 1990; Vasconcelos et al. 1990). The linamarase produced by lactic acid bacteria during fermentation of cassava roots and leaves and the endogenous linamarase contributes to the process of detoxification (Okafor et al. 1986). The hydrolysis of cyanogenic glucosides can be achieved both in the acidic environment at pH 3.8 during fermentation of cassava roots and in the alkaline environment at pH 8.5 during fermentation of the cassava leaves (Louembe et al. 1997; Vasconcelos et al. 2003; Formunyam et al. 1985)

It has been suggested that cyanogenic glycosides can control cancer cells, since these cells cannot detoxify cyanide as they do not possess the enzyme rhodanase, thus making cassava a food with health promoting properties. This cassava based therapy is still undergoing tests, but is expected to be safe as the cyanide produced is only sufficient to kill the nearby cancer cells and should any cyanide enter the blood stream, a natural enzyme produced by the liver would quickly detoxify it (Cock, 1985; Bradley, 1999).

1.3 Cheese in the African diet

The processing and utilisation of dairy products in many African countries is not well developed. The Masai tribesmen of Kenya and Tanzania who herd large numbers of zebu cattle and consume large quantities of milk have not developed a cheesemaking tradition. Nomads in developing arid countries have suffered the tragedy of hunger in recent years. It would be nutritionally advantageous if the idea of producing cheese, a stable and nutritious food which can be stored for periods of time, were introduced to tribal chiefs (Kosikowski and Mistry, 1997). Increase in the production of milk and dairy products would be a major step in improving the protein intake and ensuring a more balanced diet.

Cheesemaking in Africa is largely dictated by tradition. Due to shortage of milk, cheese production is expensive and powdered milk and cheese may be imported. The cheese produced is generally consumed very soon after manufacture, primarily because of the poor shelf life under ambient conditions. The problems are further compounded by the fact that during periods of surplus milk production the prices for milk, butter and cheese are considerably lower than in periods of lower milk production.

Rapid population growth, crippling economic problems and political turmoil in many African countries have reduced living standards and affected food availability causing widespread protein deficiencies and malnutrition. Almost half the countries of Africa experienced increases in the proportion of undernourished between 1990 – 1992 and 1994 – 96, and the poorest group of countries have not been able to reduce the number or percentage of undernourished since 1969 – 71 (FAO, 1998).

In countries with a dairy industry cheese provides an ideal vehicle for preserving the valuable nutrients of milk. Cheese is an excellent source of protein, fat and minerals such as calcium, iron and phosphorous, vitamins and essential amino acids, making it an important food in the diet of both young and old. In Nigeria, due to the lack of industrial production of traditional cheese varieties the nutritional benefits of cheese have not been utilized fully. The soft Wara cheese produced in Nigeria at farms, makes use of local ingredients. The vegetable rennet used for production of Wara cheese is produced from a native sodom apple plant (*Calotropis procera*) which can be cultivated all year round. Therefore, there is no need for imported rennet. A better understanding of the mechanism of action of this plant rennet is required if cheese production is to be carried out on a larger scale using sodom apple extract as the coagulant.

The production of cheese in African countries has been increasing this millenium from 430,000 metric tonnes in 1990 to 743,000 metric tonnes in 2002 (FAO, 2002). This increase is expected to continue as more diversification takes place and more food processing is encouraged.

Egypt has the highest cheese production of African countries and accounts for about 67 % of African cheese production (Table 4). Nigeria produced 7022 metric tonnes of cheese in 1994, and production increased slightly to 8000 metric tonnes in 2003. Most African countries increased production for total cheese (all kinds) between 1994 and 2003 except Zimbabwe that suffered a decrease in production during this period.

There is very little scientific information available on the cheeses made in Africa. Recipes and processes are passed from generation to generation by observation and practical experience. Table 5 shows the major cheese varieties produced. Even though these

Table 4. Total Cheese production (metric tonnes) in African countries

Country	Production 1994	Production 2003
Algeria	1045	2000
Angola	1007	1007
Botswana	1498	5000
Egypt	333950	498000
Eritrea	216	na
Ethiopia	4600	6000
Kenya	210	na
Mauritania	1664	2000
Morocco	6947	8000
Namibia	70	na
Niger	12064	15000
Nigeria	7022	8000
South Africa	38000	38000
Sudan	72479	152000
Tanzania	1200	3000
Tunisia	7060	14000
Zambia	1069	1069
Zimbabwe	5197	2000

(FAO, 1994; FAO, 2003) na, not available

cheeses are produced on a small scale they serve as a valuable source of nutrients and income for many small producers. However, insufficient amount of cheese is manufactured to meet the demand.

Developing countries including oil producing and exporting countries (OPEC) imported 353 000 tonnes of cheese and curds in 1994. OPEC countries alone imported 170 000 tonnes (FAO, 1994). The demand for dairy products in sub-Saharan Africa continues to increase, with the overall growth rate in the consumption of milk and milk products estimated at about 2.1% per annum. The growth in demand results from rapidly rising populations, urbanization and some increase in per capita income. This increasing demand for dairy products offers a great opportunity and potential for the low output dairy producer and provides an incentive for the development of dairy processing industry (O'Connor, 1993).

1.3.1 Egyptian Domiati

The most popular and scientifically well documented African soft cheese is Egyptian Domiati and its manufacture dates back to around 3200 B.C (Abd El-Salam, 1987).

The basic process as carried out for Domiati, a white brine pickled soft cheese, involves the use of buffalo or a mixture of buffalo and cow's milk. Buffalo milk has 7 % fat and is preferred (Scott, 1986). The milk is heated to 65.6 °C for 15 min and cooled to 35 – 40 °C.

Table 5. Traditionally produced cheese varieties in Africa

Name of cheese	Type	Source of milk	Country
Aoules	Hard	Goat	Algeria
Ayib	Soft	Butter	Ethiopia
Braided	Semi-hard	Cow, goat or sheep	Sudan
Domiati	Soft	Buffalo	Egypt
Fromage	Semi-hard	Cow	Madagascar
Fromage blanc	Soft	Skimmed	Madagascar
Gybna beyda	Soft	Cow, goat or sheep	Sudan
Karish	Soft	Cow	Egypt
Laban Rayeb	Soft	Cow	Egypt
Mashanza	Soft	Cow	Congo
Mudaffara	Semi-hard	Cow	Sudan
Wara	Soft	Cow	Nigeria
Wagashi	Soft	Cow	Benin, Mali, Nigeria
Wagassirou	Soft	Cow	Benin

(O'Connor, 1995)

It may be ripened or unripened, when ripened common salt is added up to 15% prior to rennetting. Salt may also be added to two-thirds of the raw milk and the remaining third is heated to 76.7 °C and added to the remainder to give a temperature of 35 – 46 °C, ready for rennetting (Fox, 1987; Scott, 1986). The salting prevents the growth of bacteria but delays the coagulation.

Calf rennet is used at 5 g/100 g milk and the coagulation takes between 2 – 3 h at room temperature. The curds are ladled into hoops of suitable size, e.g. 11 cm high and 12 cm diameter. The hoops are placed on a porous or coarse mat of straws. Alternatively the hoop is made long (50 cm) and wide (50 cm) with a capacity of 50 kg of curd; or factory systems use tables holding 500 kg of curd. Drainage takes 12 – 40 hours (Scott, 1986).

The pickling is carried out in large tin cans or earthenware vessels in layers. The spaces between the curds are filled with salted whey (10 – 15% salt) and the vessels are made airtight (Fox, 1987; Scott, 1986). The containers are stored at about 20 °C until required. When pickled cheeses such as Domiati are stored, it undergoes continuous proteolysis. The level of proteolysis in Domiati cheese was observed to be very high due to its high retention of milk coagulating enzymes, high moisture and salt contents (Abd El-Salam, 1987).

1.3.2 Gybnabeyda

This is a white cheese made in Sudan. It is similar to Domiati cheese made in Egypt. A starter culture is not used, and the storage life of the cheese may be over a year. The procedure for making this cheese includes heating of the fresh milk to 35 °C and followed by salt addition to give a 7 -10% salt solution in milk. Rennet or rennet extract is added to obtain a firm coagulum which develops in four to six hours. The coagulum is trans-

ferred to wooden moulds lined with muslin and the whey is allowed to drain overnight. The curd is cut into 10 cm cubes and the cubes are put into tins or other suitable airtight containers. The tin or container is filled with whey from the same cheese and sealed.

1.3.3 Ayib

This is a soft curd type of cheese made in many parts of Ethiopia. It is made from the buttermilk resulting from the churning of sour whole milk. The acidity of the used milk varies between 0.85 and 1.1% (Fox, 1987). It may also be made from skimmed milk. For making this cheese the buttermilk or skimmed milk is heated gradually to about 50 °C until a distinct curd mass is formed. Higher temperatures up to 65 °C may also be used and will result in cheese with longer shelf life. The curd mass is cooled for about one hour.

The cheese curd is separated from the whey either by ladling the curd into a separate container or by pouring the curds and whey through a sieve or fine mesh cloth and allowing the whey to drain into a container. The curds are mixed and retained in the sieve to ensure that there are no pockets of whey which could lead to off-flavours and defects in the firmness of the curd. The cheese is not pressed, but most of the whey is allowed to drain away. The cheese is stored in a clean container in a cool place. At ambient temperatures of about 30 °C the shelf life of the cheese is no more than about two days while at 4 °C it is about seven days.

1.3.4 Scarmoza

This is a pasta filata or kneaded type of cheese produced by smallholders in some regions of East Africa especially Tanzania and Kenya. Other pasta filata type cheeses are kashkaval, caciocavallo and mozzarella. About one kilogram of scarmoza cheese will be obtained from 10 litres of milk.

For this cheese fresh milk is standardised to about 3.3% fat by allowing it to settle for one hour and skimming off a portion of the fat. This fat may be used for butter manufacture. The standardised milk is heated to 36 °C and 2% of a yoghurt-type culture is added. The milk is ripened to develop acidity for 30-40 min after which the acidity of the milk should have increased by 0.02% lactic acid. Rennet is added at 1 ml per 5 litres of milk. After about 40 min the milk has coagulated and the coagulum is cut into 1 cm cubes using a sharp knife.

The curds are gently stirred with the whey mixture and they are heated to 42 °C over a period of 30 min. The curds are allowed to settle in the whey. 60% of the whey is removed from the container and the curds and remaining whey are kept at 42 °C by removing some whey and replacing it with hot whey. The acidity continues to increase giving the curd the desired texture. When the curd can be stretched into an elastic, continuous string of about one meter the correct texture has developed. The curds and whey are separated and sufficient amount of boiling water is added to cover the curd. The curd pieces are worked and folded sufficiently into a ball to give a finished cheese of about 400 g placed in a mold and allowed to cool for one hour in water at 10-12 °C. The cheese is removed from the mould and placed in a salt solution (15%) for two to three hours and ripened for about four weeks.

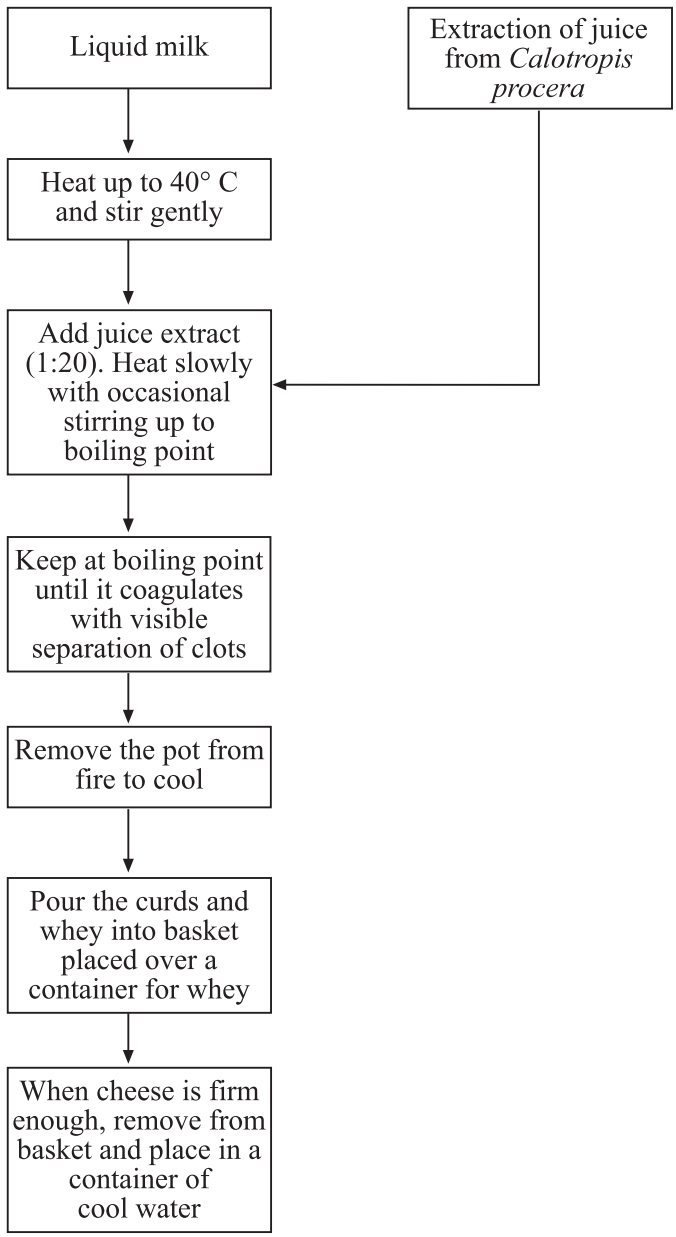

Figure 1. The traditional method for the production of Wara at farm levels in Nigeria. The manufacturing process of Wara and Wogachi are essentially the same as shown in Figure 4. The juice extract of *Calotropis procera* is often used for coagulation of the milk but juice from papaya leaves may also be used.

1.3.5 Nigerian Wara

The manufacture of Wara cheese is widespread in Nigeria and a similar cheese called Wogachi is made in the northern provinces of Benin republic, a French speaking country to the west of Nigeria. The Fulanis of Northern Nigeria are traditionally cattle rearers and they have access to fresh milk from Zebu *Bos indicus* cattles. Wara cheese making is thought to have started in this region and as a result of the normadic lifestyle of the Fulanis was spread to other parts of Northern Nigeria, Kwara, Oyo, Ogun, Ondo and the Benin republic.

Wara cheese is more common in Nigeria and size of the basket is smaller compared to that of Wogachi cheese which is more common in the Benin republic. The weight of Wara cheese obtained is about 60 g which can be sliced into smaller sizes. It is white and sold uncoloured. On the other hand, Wogachi cheese is larger, about 600 g, because the basket type mould used is about ten times bigger than the one used for Wara. Wogachi cheese is usually coloured in a hot solution of the leaves and stems of red sorghum. It may be stored for several days after it has been dipped in a salt solution for a few hours followed by immersion in the hot red sorghum solution for a few seconds.

In the traditional method shown in Figure 1, fresh morning milk is usually used. The whole milk of about 5 litres is transferred to a metal pot. The metal pot is placed over a slow burning fire or fire of smouldering wood and heated to a temperature of about 50 °C, which may take up to half an hour. The milk is stirred gently during the initial and subsequent heating and cooking, the *Calotropis procera* juice extract is then added to the warmed milk. The milk is heated slowly with intermittent stirring until it reaches boiling point. The milk is kept at boiling point until it coagulates and when there is a visible separation of curds and whey it is immediately removed from the fire.

The curds and whey are ladled or poured into baskets placed over a container for whey collection. The basket or mold facilitates whey drainage and also gives the cheese (Wara or Wogachi) its characteristic shape and size. When the cheese is firm enough to retain its shape it is removed from basket and placed in a container of cool water. The Wogachi cheese is soaked in brine (20% NaCl) for 12 - 15 hours and dipped for a few seconds in a hot solution of the stems and leaves of red sorghum.

Wara is eaten in various forms in these regions either as a normal cheese, as a flavoured snack, as a meat substitute in sauces or as fried cake or sandwich filling (Lawal, personal communication)

1.4 The mechanism of milk clotting

The main purpose of coagulants in cheesemaking is the conversion of liquid milk to a gel that can be catalysed by different proteases (Green, 1984). There are two main phases in the mechanism of milk clotting – the primary or enzymatic phase and the secondary or coagulation phase (Payens, 1982; Dalgeish, 1982; Schmidt, 1984; Green and Grandison, 1993).

Several theories by different workers on the coagulation of milk by protease enzyme have been proposed to explain this mechanism. Since 1930, Linderstrom-Lang and Holter developed a theory that the casein complex of milk owe its stability to the presence of a component that acts as a stabilizer. Rennet action starts by degrading this component specifically and the modified complex flocculates in a secondary phase (Eck, 1984).

When κ-casein, the stabilising component as part of the caseinate micelle is attacked by a milk clotting enzyme its ability to stabilize the micelle is destroyed. The Phe-Met peptide bond at position 105-106 is susceptible to this cleavage by the rennet enzyme. This reaction separates the extremely soluble hydrophilic glycomacropeptide moiety (residues 106 – 169) which will diffuse away from the micelle whereas the para- κ-casein moiety (residues 1 – 105) is strongly hydrophobic and remains on the micelle (Dalgleish, 1982). The cleavage site by chymosin at Phe105 - Met106 bond of the amino acid sequence of κ-casein is shown in Figure 2 below.

It has been indicated by experimental observations that this site is readily attacked by many different proteinases and it is probable that the bond will be cleaved by all coagulants used as rennet substitutes particularly acid proteinases with active site carboxyl groups (Fox, 1982).

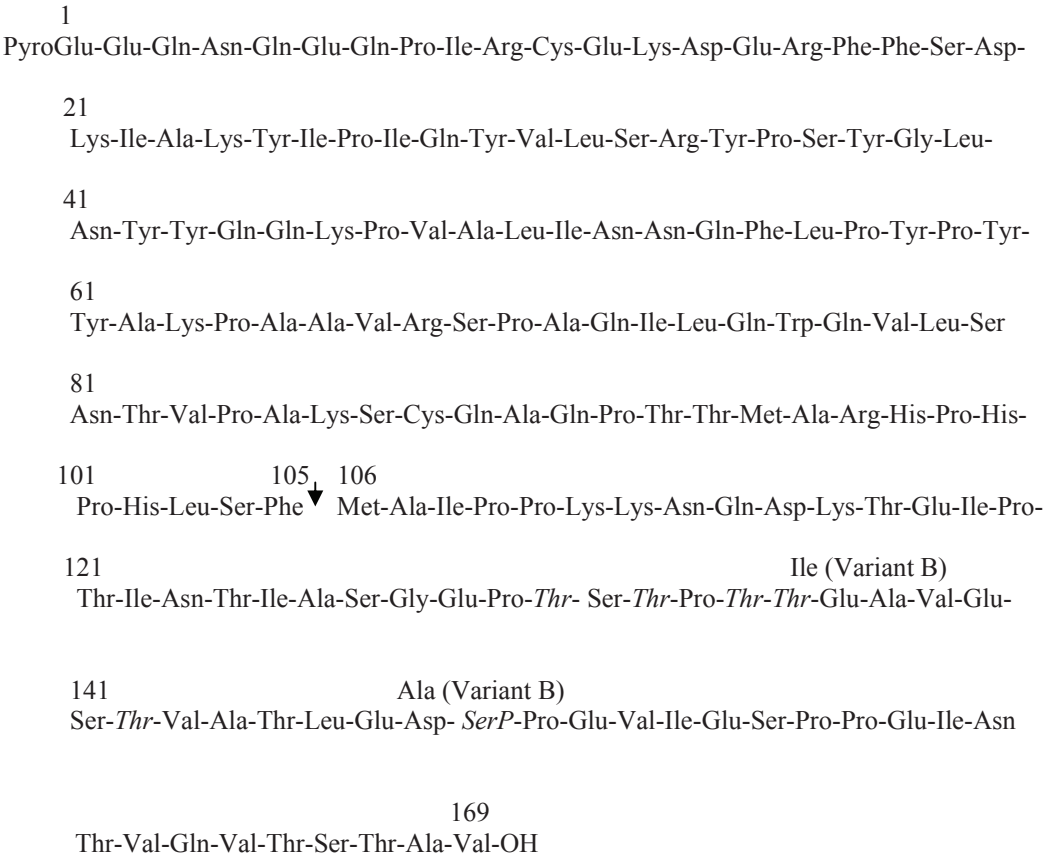

Figure 2. Amino acid sequence of κ-casein, showing the principal chymosin cleavage site (↓). Oligosaccharides are attached at some or all of the threonine residues shown in italics

However, McMahon and Brown (1984b) described milk clotting as a complex process that involves a primary enzymic phase in which κ-casein is altered and loses its ability to stabilize the remainder of the caseinate complex, a secondary non-enzymic phase in which the aggregation of this altered caseinate takes place, and a third phase where the aggregated casein micelles form a firm gel structure and possibly a final step where the curd structure tightness and syneresis occurs.

The secondary phase of coagulation is the formation of cores for the aggregation of destabilized casein micelles. The cores then grow to form milk curd resulting in the tertiary phase which is associated with an increase in the rigidity of the curd.

1.5 Microbial spoilage in soft cheese

The microbial quality of raw milk is crucial for the production of any high quality dairy food. Spoilage is a term used to describe the deterioration of a foods' texture, colour, odour or flavour to the point where it is unappetizing or unsuitable for human consumption.

The microorganisms that are principally involved in milk spoilage are psychrotrophic organisms. Most psychrotrophs are destroyed by pasteurization temperatures, however, some bacteria such as *Bacillus cereus, B. licheniformis* and *B. sporothermodurans* are able to survive and grow in subsequent processing operations during cheesemaking (Johnson et al. 1990; Petterson et al. 1996). It was observed that milk, being the only natural source of the disacharide lactose undergoes spoilage in a unique way as the lactose in milk is conspicuously utilised by coliform bacteria (Jay et al. 2005). Pasteurization of milk used in the manufacture of Domiati pickled soft cheese reduced the mean total colony count (cfu/g) by 5 log during a storage period of 30 days, coliforms and staphylococci were completely eliminated from the first day by this treatment (Salwa and Galal, 2002).

Soft cheeses have higher moisture content when compared to hard cheese and have lower shelf life due to microbial spoilage. Most soft, unripened cheeses are microbiologically unstable due to metabolic activity of bacteria, yeast or mould contaminants (Tamine and Kirkegaard, 1991; Farkye and Vedamuthus, 2002). There have been reports (Ryser et al. 1998) of some pathogenic microorganisms including *Listeria monocytogens,*and *Staphylococcus aureus* in soft cheese. *Escherichia coli* serotype 0157:H7 has been associated with the consumption of French Brie and Camembert soft cheese in the US and Scandinavia (D'Aoust, 1989).

An example of the microbiological quality of white soft cheese from hot climates is presented by Al-Mashhadi et al. (1987). They analysed the microbial quality of white soft cheese sold in three different markets in Saudi Arabia. The quality of these Saudi Arabian fresh white cheeses varied but generally high bacterial counts attributed to the processing procedures by desert dwellers were found in most samples as shown in Table 6.

Table 6. Microbiological quality (cfu $g^{-1)}$ of fresh white soft cheese sold in different markets of Madina, Saudi Arabia.

Spoilage microbes in fresh cheese	Madina markets		
	A	B	C
Coliforms	6.5×10^4	16.9×10^7	2.4×10^5
Yeasts and moulds	< 10	3.3×10^2	5.1×10^3
TCC[a]	2.2×10^6	8.8×10^8	22.4×10^7
LAB[b]	1.7×10^6	2.9×10^9	3.5×10^7
Staphylococcus aureus	<10	3.2×10^8	9.0×10^4

[a]Total colony count
[b]Lactic acid bacteria
A, B, C: Three samples each analysed in duplicate.
Adapted from Al-Mashhadi et al. (1987)

In another study on microbial quality of soft, white cheese, high counts of microorganisms were also found in the majority of the samples of Ethiopian Ayib cheese, with counts of mesophilic aerobic bacteria, yeasts and enterococci of 10^8, 10^7 and 10^7 cfu/g. About 55% of the samples were positive for coliforms and faecal coliforms, but Listeria spp. were not detected in any of the samples (Ashenafi, 1990).

Baledi or Jibnah Khadra mountain soft cheese produced in Lebanon does not preserve well and many cases of brucellosis food poisoning caused by *Brucella* spp. have been associated with it (Tannous, 1991). Another mountain cheese from Greece "Orinotyri" when made from fresh ewe's milk had a microbial count of 10^7 cfu/g for Enterobacteracea, 10^7 cfu/g for coliforms and 10^2 cfu/g for yeasts after 10 days. This was attributed to the high pH of the cheese before the action of lactic acid bacteria during ripening (Prodromou et al. 2001).

The most popular soft, white cheese named Domiati after the city and governorate of Damietta (Dumyat in the north of Egypt), is well known and widely consumed in the Arab world. The microbiology of this cheese has been very well investigated (Abou-Donia, 1986; Gilles, 1984; Nielsen, 1984). In the production of this cheese the use of starter is optional but when used it must be salt tolerant. The fresh cheese is rather salty, and as it ages it develops considerable acidity. The total counts of bacteria in fresh cheese has been reported to be 2×10^4 cfu/g as determined on standard plate count. After pickling and ripening for 2.5 months, the counts were reduced to 2×10^2 cfu/g (Rakshy and Attia, 1979a).

Lactic acid bacteria (LAB) such as lactococci and lactobacilli have been isolated from the surface slime on Domiati cheese (Abo-Elnaga, 1974a). Fahmy and Youssef (1978a) found that *Lactococcus lactis* subsp. *lactis* and *Lact. lactis* biovar *diacetylactis* were the dominant lactococci, and that *L. casei* subsp. *casei* and *L. delbrueckii* subsp. *bulgaricus* were the dominant lactobacilli in ripened Domiati cheese.

When the effect of 3 – 11% salt on the inhibition of gas forming organisms (coliforms) was studied, there was a positive relationship between the presence of coliforms and poor flavour and texture of cheese (Sadek and Eissa, 1956; Abou-Donia, 1991). Naguib *et al. (*1979a) found that *Salmonella entericia* subsp. *entericia* serotype Typhi in Domiati cheese survived up to 16 days at a level of 10% salt; El-Molla et al. (1981) reported that *S. entericia* subsp. *entericia* serotype Typhimurium survived with 5% salt added to the cheese milk but there was more inhibition at salt levels of 10% and 15%.

To reduce the microbial population in Saudi Arabian or other soft cheese they are pickled or kept in brine, which can result in mold spoilage on storage. This has led to a concern over the possibility of mycotoxin production by *Penicillium* and *Aspergillus* (Davies and Law, 1984). Another major concern is excessive salt consumption since most studies confirm that salt consumption in the African diet is on average above the recommended levels of 0.5 g NaCl per day for a healthy diet (Dillon, 1987). Therefore, natural inhibitors that have been toxicologically tested to be safe, are considered as suitable alternatives in extending the shelf life of soft cheese.

1.6 Antimicrobial effects of Lactic acid bacteria

Lactic acid bacteria (LAB) play a major part in most fermentation processes, not only because of their improving the flavour and aroma but especially for their preservative effects on food. The inhibition of food spoilage is mainly caused by the fermentative transformation of carbohydrates to lactic acid and acetic acid which lower the pH of the food thus increasing its shelf life.

In addition to acid production (acetic, lactic and carbonic), LAB contribute to preservation by production of a vast array of antimicrobial compounds and proteins (Ray and Daeschel, 1992, Elliason and Tatini, 1999, O'Sullivan et al. 2002). LAB can adapt to various conditions and change their metabolism, they are able to degrade different carbohydrates and related compounds to yield antimicrobial substances, e.g. *L. reuteri* dehydrates glycerol to accumulate and excrete the intermediate 3-hydroxypropionaldehyde (3-HPA) or reuterin which is a potent antimicrobial substance (Talarico et al. 1990). Sour dough lactic acid bacteria have been reported to produce inhibitory substances such as bavaricin A, plantaricin ST31, reutericyclin and bacteriocin like inhibitory substance BLIS 57, which would be of benefit in preventing ropiness mainly caused by *Bacillus* species in bread (Rosenkvist and Hansen, 1995; Messens and De Vuyst, 2002).

Other low molecular mass antimicrobial substances include diacetyl, acetaldehyde, hydrogen peroxide, lactoperoxidases naturally occurring in milk, and various inhibitory substances produced by *Lactobacillus casei* (Lortie et al. 1993). Low molecular mass 2-pyrrolidone-5-carboxylic acid (PCA) also known as pyroglutamic acid has been found to contribute to the antimicrobial activity of *L. casei* ssp. *casei* LC-10 and *L. casei* ssp. *pseudoplantarum* LB1931 and they showed inhibition towards *Bacillus subtilis*

1205, *B. subtilis* MCM-1, *Enterobacter cloacae* 1575 and *Pseudomonas putida* 1560-Z (Huttunen et al. 1995). It has also been reported that low molecular mass of new types of antimicrobial compounds identified in the culture filtrate of *Lactobacillus plantarum* VTT E-78076 totally inhibited the growth of gram-negative test organism *Pantoea agglomerans* (*Enterobacter agglomerans*) VTT E-90396 (Niku-Paavola et al. 1999). Bacteriocins are antimicrobial proteins or oligopeptides that are active against bacterial strains closely related to the producer strain (Jung, 1991a). The first antibacterial peptide discovered in lactic acid bacteria was reported in 1928 by Rogers

who observed an inhibitory substance later named nisin from *Lactococcus lactis* strains. Unlike most bacteriocins, nisin has a broad inhibitory spectrum (Jack et al. 1995). It consists of 34 amino acid residues (3,352 Da) including one lanthionine (3,3'-thiodialanine), four β–methyllanthionines, one dehydroalanine and two dehydrobutyrines (Jung, 1991). Due to the unusual lanthionines nisin is classified as belonging to lantibiotics and due to its elongated structure to the type A group, class I (Klaenhammer, 1993).

The first natural variant, nisin Z was presented at the first international workshop on lantibiotics and related modified antibiotic peptides by Graeffe et al. (1991) and Kuipers et al. (1991). Mulders et al. (1991) also showed that the nisin Z variant is more widespread when nisin producers were analysed. Nisin Q variant was isolated from a *L. lactis* strain isolated from a Japanese river and has three amino acid differences compared to nisin Z (Zendo et al. 2003). Recently, a strain of *Streptococcus uberis* was found to produce nisin U and differs by nine amino acids when compared to nisin A and Z (Wirawan et al. 2006).

Toxicity studies for nisin have been carried out using amounts far in excess of the amount that would be used in foods with no ill effects (Adams and Smid, 2004). Nisin is rapidly inactivated in the intestine by digestive enzymes and is undetectable in human saliva ten minutes after consumption (Abee et al. 1995). Its non-toxic nature and natural presence in many fermented foods helped to get it approved for use in foodstuffs by food legislation bodies in the US and the EU (Hurst, 1981; Thomas et al., 2000). Due to its ability to strongly inhibit the outgrowth of spores and the growth of a broad range of gram-positive microorganisms, nisin is used in the food and dairy industries for safety purpose and to increase the shelf life of processed cheese, dairy desserts, milk, canned foods, meat, eggs, fish and alcoholic beverages (Delves-Broughton, 1996; Heller, 2001; O'Sullivan et al. 2002). In addition to inhibition of the growth of food borne pathogens and food spoilage organisms such as *Bacillus cereus, Clostridium botulinum, C. perfrigens, Listeria monocytogens, Staphylococcus aureus* (Fowler and Gasson, 1991; Delves-Broughton et al., 1996) nisin can inhibit gram negative bacteria such as *Salmonella* species if the outer membrane of the target cell is first destabilized with EDTA (Stevens et al. 1991; Fowler et al. 1991; Alakomi et al. 2000).

Lactic acid bacteria have the capacity to produce a wide range of bacteriocins (Hugenholtz et al. 2002, Twomey et al. 2002, Hanniffy et al. 2004). All bacteriocins have a protein or a peptide component that are essential for their bactericidal function, some were reported to consist of combinations of different proteins or are composites of proteins together with lipid or carbohydrate moieties (Schved et al. 1993). Many of the bacteriocin producers have been isolated from fermented foods and are evaluated for their potential of preserving food and increased safety.

The non-lanthionine bacteriocins are generally classified as class II and III groups (Klaenhammer, 1993). The class II contains the heat stable, small peptides (< 10 kDa), while class III are heat labile and relatively large at 20 kDa (Nes and Holo, 2002). Some potentially useful non-lanthionine bacteriocins in food applications include sakacin A and P from *L. sake* Lb 706 isolated from raw meat, leucocin A from *Leuconostoc gelidum* VAL 187 for processed meat*,* enterocin A from *Enterococcus faecium* CTC 492 for fermented sausage, and pediocin Ach from *Pediococcus acidilactici* H for fermented meat (Axelsson et al. 1989; Chung et al. 1989; Talarico et al. 1990, Ray et al. 2001b). Other bacteriocins from foods associated with lactic acid bacteria that have been identified and characterized include diplococcin, acidophilin, bulgarican, lactacins, and plantaricins (Nettles and Barefoot, 1993; Araceli et al. 2003; Chen and Hoover, 2003).

Nowadays consumers demand purer and safer food with a preference for foods with less chemical additives. As a result there is a continuous search for natural inhibitors of unwanted microbial growth. In this respect bacteriocins such as nisin are important and promising, since they may offer a better alternative to chemical food additives in the control of pathogenic organisms in food (Salminen and von Wright, 1993).

Most traditional African foods are fermented before consumption. *L. plantarum* F1 and *L. brevis* OGI were isolated from Nigerian fermented food products such as ogi and cassava. They produce bacteriocins that has a broad spectrum of inhibition against pathogenic food spoilage organisms (Ogunbanwo et al. 2003; Odunfa et al. 1996). The shelf life of ogi, a fermented cereal based porridge was extended by four days and the growth of *Escherichia coli* was retarded from log 6.41 to log 1.7 after 6 h incubation, when a bacteriocin producing *Lactobacillus* strain was added during production (Olasupo et al. 1997). The occurrence of nisin Z in many strains of *Lactococcus lactis* from different origins has been reported (Graeffe et al. 1991; Kuipers et al. 1991; Cai et al. 1997). The first report on nisin Z in African traditionally produced foods was in Nigerian Wara cheese (Olasupo et al. 1999). A bacteriocin producing LAB strain, *Enterococcus faecium* NA01 isolated from Wara was also shown to be inhibitory towards *Lactobacillus, Enterococcus* and *Listeria* strains (Olasupo et al. 1994).

2 AIMS AND OBJECTIVES OF THE STUDY

The overall aim of this dissertation was to raise awareness on the application of technology and microbiology on African foods such as cereal porridge, cassava and Wara cheese. A thorough understanding of these foods hopefully will yield novel food products that will benefit the African population.

The specific investigation on the utilization of *Calotropis procera* as a coagulant in the production of Nigerian Wara cheese aimed:

- to study the coagulation of milk by a protease preparation from *Calotropis procera* and determine its mode of action
- to estimate the molecular weight of the protease enzyme
- to optimise methodology for the production of Wara cheese and improve its overall acceptability
- to investigate the application of nisin producing bacteria as starter cultures for use in Wara cheese production.

3 MATERIALS AND METHODS

3.1 Materials

Milk (fresh and semi skimmed) was from Valio Limited Finland, soymilk from Yeo Hiap Seng of Malaysia and coconut milk from Coconut and Allied Products, Sdn Bhd Selangor Darul, Malaysia. *Calotropis procera* leaves were harvested in the early morning hours from Lagos, Nigeria. Cassava gari was processed in Ghana, marketed by Akoma Foods, UK and Nigerian processed gari was marketed by Wazobia Foods, USA.

3.2 Methods

Table 7. Methods employed in this study

Method	Description	References
Viscosity of cereal starch	Brabender viscograph E	I
Nisin on cassava gari	Effect of nisin on *Bacillus subtilis* BRB1 grown in Luria media	Sibakov et al. 1983,
Thermal and pH stabilities of plant rennet	The optimal temperature and pH of *C. procera* coagulant	III
Rennet strength	Rennet strength of *C. procera* coagulant	III
Extraction and purification of protease in *C. procera*	$(NH_4)_2SO_4$, acetone precipitation, centricon ultrafiltration & FPLC	III
Mol. weight determination	SDS-PAGE & Zymography	Andrews, 1983, Leber and Balkwill, 1997, III
Proximate analyses of Wara	moisture, fat, protein, lactose and ash determinations	AOAC, 1995
Effects of cations on milk clotting	section 3.2.3 below	Berkowitz-Hundert et al. 1964, unpublished
GFP-based nisin bioassay	Fluoroskan Ascent 374 scanning fluorometer computer-linked with Ascent version 1.2 software (Lab-Systems, Helsinki, Finland)	Reunanen and Saris, 2003, IV
Hedonic rating	Sensory evaluation	Piggott, 1984, IV

3.2.1 Action of nisin in cassava gari samples

Gari prepared from fermented cassava in Nigeria and Ghana, 5% (w/v) were autoclaved before being spiked with *Bacillus subtilis* BRB1 bacteria spores grown in Luria media at 37 ºC overnight (Sibakov et al. 1983). The inhibitory action of nisin at 10, 100 and 500 IU were tested on the outgrowth of *B. subtilis* on gari samples.

3.2.2 Laboratory production of Wara cheese

Milk samples, (200 ml) in a 250 ml volumetric beaker were heated to a temperature of 40 °C in a water bath. Coagulant (10 ml) was added and the milk was then left until a clot had been formed. The beaker was transferred to a hot plate, the temperature was raised to 80 °C and then held at this temperature for 10 min. During this period the curds released its whey until curd formation that resembles the traditionally produced Wara cheese was achieved. The whey was drained from the curds using a tea sieve lined with cheese cloth.

A starter mediated production of Wara cheese by nisin producing lactic acid bacteria *Lactococcus lactis* LAC309 was employed to extend the shelf life of Wara (paper IV).

3.2.3 Effects of cations on the milk clotting activity

The effects of divalent (Ca^{2+}, Mg^{2+}) ions (from calcium chloride dihydrate and magnessium chloride hexahydrate, respectively) and monovalent (Na^{+} and K^{+}) ions (from sodium chloride and potassium chloride respectively) were tested on the clotting ability of the extract. Varying amounts of the salts (in milligrams) were added to 2.5 ml of milk, the pH was adjusted from 6.7 to 5.1 or 5.9 by 0.1 M HCl and incubated at a temperature of 70 °C in a water bath. An aliquot of 250 µl of the *Calotropis procera* leaf extract was added to the mixture in test tubes, and the time taken for clot formation was noted.

4 RESULTS AND DISCUSSION

4.1 Transfer of Technology (I)

In line with the United Nations Millenium Development Program, it was recognized as of vital importance that the availability of African foods be improved with technological inputs to the raw food crops. During the period 1990 to 1997, manufacture value added in sub-Saharan Africa excluding South Africa grew only by 0.1 % per year (UNIDO, 1999).

A common pattern in the African food and nutritional crisis is lack of commitment to implement realistic visions and strategic policies to guide the agricultural and food sectors. Most of the prescriptions needed to move Africa from its chronic food crisis to accelerated food production, processing, and diversification, are documented in various resolutions passed in many international conferences but delivery is a challenge.

The transfer of technology from the North to South should to take into consideration the inherent challenges peculiar to a country to avoid the risks of running "white elephant" developmental projects as discussed in (I). African governments need to pay attention to and begin acting on the advice of their own private sectors, research and development experts, policy analysts, technology practitioners, the entrepreneurial and industry community, as well as the traditional community leaders who know about strategies for community-based food and nutritional security. These should be tackled as a first line of security against cyclical food scarcity and malnutrition.

Local initiatives when properly harnessed resulted in a successful implementation of the Mtwara-Lindi water project in rural south-east of Tanzania in East Africa (Johansson and Mlenge, 1993; I). Water shortage contributes to poverty and starvation and has been identified as a serious constraint to industrial development (McLaughlin et al. 2004). The importance of clean and easily accessible water is vital to the environment, agriculture, health and sustainable development in Africa and other developing countries. Water scarcity and the consequent environmental problems can threaten an entire agricultural production systems as well as human and natural life systems (Zehnder et al. 2005).

African countires need to industrialize efficiently in order to achieve growth and competitiveness and reap the benefits of modern technology. This will require new organizational methods, technological change, flexible response, greater networking and closely integrated systems across regions (UNCTAD, 2003, UNIDO, 2004).

The transfer of technology is vital for today's globalized world. The changing global scenario has affected the competitiveness of countries which has led to intensification in the process of international flow of technologies across national boundaries, increased technological cooperation, strategic alliances and partnership (APCTT, 2005).

4.2 Applied research and developments on cereal porridge (II)

Cereal porridges are often some of the first complementary foods to be introduced in the diets of weaning infants in developing countries (Nout, 1993). The World Health Organization recommended the introduction of complementary foods in addition to human milk at 6 months of age in order to improve the chances of survival of young children (Underwood and Hofvander, 1982; WHO, 2003).

Pasting properties of flours or starch are influenced by several factors such as granule size, structure, amylose/amylopectin ratio and molecular weight (Thomas and Atwell, 1999; Mariotti et al. 2005). The consistency and dry matter of cereal porridge can be controlled by amylolytic enzyme treatment in the cooking stage (Salovaara, 1991).

The utilization of porridge made of any cereal grain by infants would require a lower consistency, obtained for example by amylase enzyme treatment to yield higher energy values. Recent attempts to hydrolyze *Sorghum bicolor* starch with amylase from *Rhizopus* sp. showed the optimal temperature and pH for the activity of the amylase enzyme from this fungus to be 55 ºC and 5.0. The gelatinized sorghum starch has to be cooled to ensure a maximal hydrolytic action of the amylase (Adebiyi et al. 2005).

In my studies the usefulness of amylase treatment to decrease the viscosity of cooked and fermented oat porridge was tested (II). Oat cereal was investigated rather than an African cereal for the fact that oat is readily available in Finland and the project was partly financed by JVS foundation as part of an extensive research to promote oat utilization in Finland. The pasting curves of 10% oat bran porridge cooked in a viscograph with malt flour addition had a gelatinization temperature of 77 ºC with a peak height of 302 Brabender units (BU) which was significantly reduced to 57 BU with 0.5% malt flour addition (II). This reduction was more than 70%, which makes it ideal for pumping in industrial production. Treatments with amylase lowered the viscosity making it possible to increase the solids content of oat bran porridge without compromising its ability to flow. Jansson and Lindahl (1991) also considered it an economic advantage to have a higher concentration and yet maintain fluidity in pumping or storing of oatmeal suspensions in a large scale operation.

The pasting properties of cooked and fermented traditional Beninese ogi was shown to be similar to those obtained in previous works for sorghum or maize ogi and mawe (Adeyemi et al. 1987, Hounhouigan et al. 1993a). Ogi pastes made with 10 % dry matter had a gelatinisation temperature of 71.6 ºC and a peak height of 936 BU (Adeyemi and Beckley, 1986, Nago et al. 1998). For comparison with other starches in industrial application, tapioca starch from cassava gelatinizes at a temperature between 59 - 65 °C and has a slightly lower viscosity than waxy maize (Obanni and Be Miller, 1997).

An important criterion in the development of cereal porridges in African countries is nutritional quality. Substantial nutrient losses occur during the various traditional steps of ogi processing. Much of the protein in cereal grains is located in the testa and germ, which are usually sifted off during processing. These losses have been evaluated and reported by several workers (Hamad, 1978; Oke, 1967).

One major nutritional problem with cereal based complementary foods is phytic acid in cereals, which inhibits iron absorption. Low iron absorption from cereal porridges contributes to the high prevalence of iron deficiency in infants from Africa and other developing countries, which results in anaemia, retarded psychomotor and mental development (de Andraca et al. 1997, Hurrell et al. 2003).

The bioavailability of iron can be improved in complementary cereals and legumes by either mixing the cereal with ascorbic acid rich foods or by simultaneous consumption of these foods, by activating the native phytases by commercial enzyme or by a combination of soaking, germinating and fermentation (Davidson, 2003; Müller and Krawinkel, 2005).

Recent development on the utilization of amylase enzymes to lower the viscosity of African cereal porridges such as Zoom-koom, dĩgue and dolo from Mali showed that three West African plants degraded starch and allowed infants to consume porridges with high energy intake. The leaves of *Boscia senegalensis* contain endo-1,3- β-glucanase, an unusual enzyme with respect to its ability to degrade insoluble yeast glucans and yield valuable oligosaccharides. Zhizomes of *Curculigo pilosa* contain β-amylase which degrades raw starches from wheat, corn, potato and rice. In the bulbs of *Gladiolus klattianus* there are activities of α- and β-amylases (Dicko et al. 2005)

Apart from the industrial benefits when viscosity is reduced by amylase treatment, fermentation of cereals was observed to improve their nutrition values in traditional diets (Chavan et al. 1989).

In the fermentation of Nigerian ogi from maize, it has been shown that *Lactobacillus plantarum* was the predominant organism responsible for lactic acid production and *Corynebacterium* hydrolysed the maize starch to carboxylic acid while *Saccharomyces cereviseae* and *Candida mycoderma* contributed to flavour development (Odunfa, 1985).

A further improvement on the nutritional quality of ogi is the manufacture of soy-ogi, in which 10 % soy flour is added during production by the Federal Institute of Industrial Research, Oshodi (FIIRO) in Nigeria (Haard et al. 1999). A microbiologically safe, oat based fermented yoghurt Yosa™ has been formulated and patented and is marketed with health benefit effects (Bioferme, 2004).

A cooperative research network designed to upgrade the technology for large scale industrial production of fermented cereals in Nigeria and other African countries has been recently advocated (Achi, 2005). Nigerian cereals can be developed on a large scale industrial production into a spoonable, fermented, non-dairy yoghurt like product by amylase treatment and lactic acid bacteria inoculation similar to oat based Finnish Yosa.

4.3 Applied research and developments on cassava products (I, III, and additional unpublished results)

Cassava has been shown to be a great hope in reducing hunger and poverty in developing countries (III, Onabolu and Bokanga 1998). The high carbohydrate content of cassava roots, and the high protein and micro-nutrient of cassava leaves make them suitable for livestock feed as they have been shown to increase milk production of dairy cows (Ntawuruhunga, et al. 2004).

Researchers are striving to increase and stabilize yields from cassava to resist major pests such as the biological control of mealy bug plague and emerging diseases such as cassava brown streak and mosaic caused by virus, cassava bacterial blight caused by *Xanthomanas campestris* pv. *manihotis* (Manyong et al. 2000).

There have been concerns on the toxicity of the cyanogenic glucosides in cassava (III, Kobawila, 2005). Efforts have been made during the traditional processing by fermentation and heating of food products from cassava to ensure adequate breakdown of the glucosides (III, Okafor and Ejiofor, 1986). In Central Africa, the spontaneous fermentation of cassava roots in foo-foo production has been shown to degrade the endogenous cyanogens almost totally, plant cells were lysed by the simultaneous action of pectin methylesterase and pectase lyase leading to the production of organic acids (Brauman et al. 1996).

The ability of nisin to inhibit the outgrowth of *Bacillus subtilis* BRB1 spores spiked gari samples from Nigeria and Ghana was tested. Nisin at 500 IU/ml was sufficient to eliminate outgowth of the *B. subtilis* spores in both samples. Lower level of nisin resulted in spore outgrowth (results not shown). This suggested that nisin could be used to improve the safety and shelf life of gari similarly as nisin has been shown to be useful for spore inhibition in a variety of other food products (Caplice and Fitzgerald, 1999; O'Sullivan et al. 2002).

The application of cassava or tapioca starch in food industries as a food ingredient in developed countries has been shown to be on the increase, tapioca starch has a good mouth feel, and is often used in the food industry for thickening (ISI, 2005). The pasting or gelatinization temperature in acid-thinned cassava starch was found to increase with increase in acid hydrolysis. Acid modification of cassava starch at 60 ºC with 0.1 M hydrochloric acid solution for 1 h and 2 h gave pastes that are stable with workable viscosities and can form a gel in food processing (Ahmed et al. 2005).

Cassava starch can be converted to maltotriose, maltose and glucose as well as to other modified sugars and organic acids (Tan et al. 1984, Vuilleumier, 1993). It can also be applied in extruded snacks, where it improves expansion, custard-type pie filling, where it reduces surface cracking and in baby foods as a bodying agent. In biscuits and in cream sandwiches 5 - 10 % tapioca starch softens the texture and renders the biscuit nonsticky (ISI, 2005).

In Malaysia, cassava starch and roots are used to produce dried yeast and alcohol industrially. A volume of 70–110 litres absolute alcohol can be obtained per tonne of cassava. The production of L-lactic acid from cassava starch in a bioreactor using *Aspergillus awamori* and *Lactobacillus lactis* spp. *lactis* has been demonstrated (Roble et al. 2003). Hong et al. (2001) showed that cassava dregs could be employed for phytase production after the addition of a nitrogen source and mineral salts and cassava peels could be used to make activated carbons which are efficient as adsorbents for dyes and metal ions (Rajeshwarisivaraj et al. 2001)

Vitamin A deficiency has been reported to result in xerophthalmia and tragic blindness in at least a million children every year (Hegsted, 1978). An improved yellow tuber cassava germplasm with higher amounts of β-carotene increases the levels of provitamin A compounds which would mean healthier immune system, better eye health and less blindness (Owor et al. 2004, Ntawuruhunga et al. 2004). The International Centre for Tropical Agriculture (CIAT) based in Cali, Colombia collaborates with the International Institute of Tropical Agriculture (IITA) based in Ibadan, Nigeria to reach out to several African countries as part of the Global Cassava Development Strategy with new biotechnical techniques such as molecular tagging of genes controlling traits and for marker assisted selection (CIAT, 1993; Wenham, 1995). A major development with financial support from the Melinda and Bill Gates Foundation to develop new varieties of cassava plants that will have increased levels of zinc, iron, protein, vitamins A and E is relevant to the lives of millions in African countries and they should benefit from such concerted efforts by concerned institutions (I).

During the field work conducted in Nigeria, I organized local workshops and gave lectures to different groups, schools and non-governmental organizations on the relevance of cassava in the society (III). The National Fadama Development Project and

post-harvest unit of the IITA were cooperative during the field and study visits (unpublished report). IITA research knowledge and products developed are for the benefit of people in the developing world, the Integrated Cassava Project (ICP) is a laudable project that should be encouraged by national agencies to ensure that the novel value added cassava products reach the poor masses.

The utilization of research findings from the North to grassroot people in the South will depend on getting these people actively involved. My field works in Nigeria and experience from the water project in Tanzania recognised the need to emphasize research that will have direct impacts in transforming the lives of these societies (I). A recent successful utilization of cassava in Nigeria by IITA/ICP is the production of glucose syrup in which amylase treatment was used to liquefy the gelatinized starch in cassava, similar to reducing the viscosity of cooked oat bran (II) but the cassava starch was hydrolyzed very rapidly to produce dextrins by Termamyl 120 L α-amylase. When liquefied, the pH was reduced to 4.2 - 4.5, and the solution was cooled to 60 ºC. A glucoamylase (Novo's AMG) is added immediately. The enzyme releases single glucose units from the ends of dextrin molecules leading to the production of 95 % glucose (IITA/ICP, 2005).

4.4 Applied research and developments on Wara cheese (IV and V)

4.4.1 The Characterization and Application of *Calotropis procera,* a coagulant in Nigerian Wara cheese (IV)

The protease extracted from *Calotropis procera* leaves, a coagulant in the traditional production of Nigerian Wara cheese was characterized and partially purified (IV). Results showed optimal activities at high temperature (70 ºC) and at pH 5.6, which makes it ideal for Wara commercial production, where higher turnover is desired and no ripening is required.

The specific activity of the purified protease enzyme in *C. procera* coagulant increased progressively from the crude extract at 0.107 units/mg to 2.933 units/mg at the final step of purification by Fast Protein Liquid Chromatography (IV).

Milk clotting action was enhanced strongly by divalent ions calcium chloride and magnesium chloride. However, with magnesium chloride, when the concentration was above 85 mg, an inhibition was noticed and the clotting time increased i.e no clotting occurred before 30 min (unpublished). Berkowitz *et.al*. (1964) observed that the weak clotting action of vegetal and animal chymosin can be enhanced by divalent cations but at lower pH values the activating effects was limited and turned into inhibition at high ion concentrations. Slorza-Feria (2001) showed that divalent cation increased the calcium concentration in milk thereby influencing the clotting time.

There were higher yield of protein, ash, iron and vitamin A in Wara cheese when a starter culture of Lactococcus lactis was employed in comparison to market samples (Sanni et al. 1999).

4.4.2 Inhibition of toxicogenic *Bacillus licheniformis* 553/1 in Nigerian Wara soft cheese by nisin producing *Lactococcus lactis* LAC309 (V)

When a starter lactic acid bacterium *Lactococcus lactis* LAC309 was added to laboratory produced Wara cheese, there was an inhibitory effect on *Bacillus licheniformis* after 3

days. The *L. lactis* LAC309 containing Wara cheese had 2.9 x 10^4 cfu of *B. licheniformis* compared to 1.65 x 10^8 cfu of *B. licheniformis*/g in Wara cheese without addition of *L. lactis*. To extend the shelf life of Wara cheese in large scale commercial production, a nisin producing starter would be ideal as it is safer and more palatable (V). The enzymes from *Lactococcus lactis* starter cultures was shown to degrade caseins which led to the formation of key flavour components that contributed to the sensory perception (palatability) in cheese (Smit et al. 2005).

5 GENERAL DISCUSSION AND CONCLUSIONS

It has been very difficult to obtain information on non-European cheese varieties since only limited published data are available and much of it is rather superficial compared to what is available on the major European varieties. This research relied on the few publications on Nigerian Wara soft cheese and personal communication where appropriate.

The processing of Wara cheese in Nigeria can be industrialised based on the application and characteristics of the protease enzyme from *Calotropis procera*. However, a regular supply of milk will be vital for the establishement of such industries. Since the processing of Wara cheese does not involve ripening, the production output is quite fast and there would be large scale production with minimal costs to the cheesemaker.

Attempts were made to standardise and purify the enzymes from *Calotropis procera* in the laboratory production of Nigerian Wara. The use of lactic acid bacteria starter mediated for its production also proved successful. In many African countries, cheeses are produced in small quantities, in many cases defined starters are not used so that acid production by indigenous microflora or whey from previous batches is probably rather variable (Fox, 1987).

The molecular weight of the partially purified protease of *Calotropis procera* as estimated by the zymogram and sodium dodecyl sulphate polyacrylamide electrophoresis in this research was around 60 kilodaltons. The utilisation of a plant vegetable rennet as an alternative to standard calf rennet is promising especially when soft, unripened Wara cheese were made. In the attempts to purify and determine the amino acid composition of the protease enzyme in *Calotropis procera* for milk coagulation there were drawbacks. Some of the drawbacks with plant cells when utilised are the nature of the highly compartmented cells and it was shown that the bulk of the volume is vacuolar space which can be filled with quite acid solution, proteases, and a variety of detrimental compounds. Consequently plant extracts may be low in protein (Scopes, 1984). The abundance of chloroplasts in leaf cells coupled with the semiautonomous character of their metabolism presents a special problem. Numerous cytoplasmic and nuclear biochemical pathways are partially duplicated in the chloroplast such that a given enzyme assay may detect both cytosolic and chloroplastic activities (Dalling, 1986).

Another problem with plants, particularly during preparation of the crude extract for purification is their phenolic compounds content which oxidize mainly under the influence of endogenous phenol oxidases to form dark pigments. These pigments attach themselves to proteins and react covalently to inactivate many enzymes. Some phenolics are frequently present in bound form and constitute either complex structures like hydrolysable tannins or simple molecules by combining with sugars or organic acids (Macheix *et al*. 1990). This problem may be solved by the inclusion of a thiol compound such as β-mercaptoethanol which will minimise the action of phenol oxidases or the addition of powdered polyvinylpyrolidine which adsorb the phenolic compounds.

Suggestions

On the basis of evidence obtained from the field and laboratory investigations on cereal porridge, cassava and the application of *Calotropis procera* in the production of Wara soft cheese the following are suggested:

Cereal porridge developments:

- Liquefaction by amylase treatments on local grains will lead to the formulation of novel cereal based products with added health and safety benefits.

Cassava based products developments:

- National research agencies supported by the government should promote the commercialisation of end-use products from cassava

Wara cheese developments:

- The possibility of improving the cheese flavour with exotic tropical fruits which are readily available in Nigeria such as gauva, mango, pineapple e.t.c will make the spreadable soft cheese more acceptable in the Nigerian market.
- The structure of the enzyme when fully purified will need to be determined in detail so that the amino acids sequence can be elucidated enabling cloning of the corresponding gene. Then, the protease could be more easily produced, enabling economical usage of it as a rennet.
- The use of a natural preservative such as nisin producing *L. lactis* LAC309 in the traditionally produced cheese need to be promoted.

Additionally, it is important to emphasize that:
- The transfer of technology from developed countries to African countries should accommodate local initiatives and cooperate with existing institutions to reach the grassroots.
- The establishment of more cottage industries, incorporating technological and microbiological applications to locally grown cereal crops, cassava root tubers and milk will reduce post harvest waste. This will eventually help to provide more jobs, alleviate hunger and poverty.

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"And half a grain of reality like the smallest portion of some scarce natural productions will flavour an enormous quantity of diluent"
--From Little Dorrit, Charles Dickens

"Anayekula ameonja ugumu wa kazi. (Swahili)
The one who eats has tasted the hardship of labour. (English)
Ne mange que celui qui a gouté à la fatigue du travail. (French)"
--Native African proverb

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